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Research Paper

Water purification system microbiological evaluation in health care environment

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Abstract

Purified water is used in the production of commercial preparations, pharmaceutical applications, cleaning of semi-critical devices, areas and equipment, as well as in the preparation of pharmaceutical chemicals and bacteriological media. The maintenance of water purification systems requires routine cleaning and disinfection of each stage of the system to remove accumulated particulate matter and to prevent bacterial growth. Contaminants commonly found in feed water decrease the efficiency of the system and increase the cost of producing purified water. The water purification system used in this study comprises four main steps: pretreatment, purification, storage and distribution of purified water. Total coliform viable counts were enumerated in minimal R2A agar and m-Endo agar medium. Cleaning and sanitization of the purification system stages were performed periodically, or when the system was stopped longer than 24 hours. The contamination of water was verified in all the treatment steps for the system studied, especially in the activated carbon. The most part of contamination was eliminated by reverse osmosis. These process generate 40% waste water. In order to take the advantage of these waste waters, the Pilot Unit Beneficiation Water (WBPU) has been proposed. It plans to apply ultraviolet radiation and ozone gas to eliminate contamination from waste waters, rather than the chlorination, which damage the reverse osmosis membrane. These waste waters can be used to mix with the rain waters to pH values adjust.

Keywords: dialysis, reverse osmosis, water beneficiation, water contamination, water system, waste water.

Introduction

Purified water is used in the production of commercial preparations, pharmaceutical applications, cleaning of semi-critical devices, areas and equipment, as well as in the preparation of pharmaceutical chemicals and bacteriological media[1]. Poor water quality continues to pose a major threat to human health causing severe diseases. Diarrhea alone amounts to an estimated 4.1 % of the total daily global burden of disease and is responsible for the deaths of 1.8 million people every year. It was estimated that 88% of that burden is attributable to unsafe water supply, sanitation and hygiene and is mostly concentrated on children in developing countries[2]. The bacterial contamination of solutions and fluids in health care environments has been reported [3,4,5] as a public health problem and it may be caused by the water used for the solution preparations. It is reinforced the need for complete guidelines regarding the water purification system that include relevant chemical and microbiological parameters. Drinking water[6], when used in pharmaceutical formulations, must be purified, according to mandatory requirements for purified water, water for injection or sterile water[7].

The maintenance of water purification systems requires routine cleaning and disinfection of each stage of the system to remove accumulated particulate matter and to prevent bacterial growth [8]. Contaminants commonly found in feed water decrease the efficiency of the system and increase the cost of producing purified water[9,10]. The systems are usually equipped with reverse osmosis that requires pretreatment of the incoming water, which adds significant costs to their operation and maintenance. Nevertheless, these procedures are mandatory to guarantee the effectiveness of the system in removing low molecular weight organic elements[11], including pyrogenic particles and microorganisms. From osmosis reverse and distillatory stages, there are water waste of 35% to 60% v/v that can be a safe source of water that treated should ensure appropriate potability[12,13]. An alternative source treated recycled water from osmosis reverse and distillatory, as well as, rainwater that can be appropriated captured, stored and purified.

Searching for continuous improvement of the process to produce purified water, the present work aimed evaluate the cleaning and sanitization procedures, applied to the water purification system, through microbiological analyses, which allowed identify critical points of contamination during the treatment. Furthermore, the work involved the collection and analysis of data from microbiological evaluations conducted at every step of the purification system (Water Benefit Pilot Unit - WBPU) and identification of critical points of bacterial contamination, in order to set maintenance and disinfection of the supplier stages and prevent recontamination.

Materials and Methods

Purification System

The water purification system used in this study comprises four main steps: pretreatment, purification, storage and distribution of purified water, in Table 1. The sampling points for the microbial analysis follow the water flow through the system.

Table 1: Flowchart of the water purification system

Stages of water purification system		Sampling points
Pretreatment	Municipal water inlet	I
	Multimedium filter	II
	Softener	III
	Charcoal filter	IV and V
Purification	Reverse osmosis	VI
	Deionizer	VII
	Storage tank	VIII
Storage and distribution	UV lamp	IX
	0.05µm filter	X
	Consumption - Leakage test	XI
	Consumption - automatic washer*	XII
	Consumption - wash basin*	XIII

*Automatic washer and special wash place for oxygenator components, in a clean room (class 100)

In the purification process performed through the U.S. Filter system (U.S. Filter, Lowell, MA, USA), municipal water (I) from the public water supply system was stored in a 10,000 L fiberglass reservoir. A pressure pump, with flow of 34 L/min and a pressure of 413 kPa, pressurizes the water through the purification system. A volume of 3500±500 L/day water is routinely purified through the system. The purified water is applied for washing thermoplastic components, which are used on the assembly line of the oxygenator, and, it is also used in tightness tests for detection of possible leakage in the finished product.As the municipal water enters the purification system, it flows through two multimedia filters (II) composed of anthracite, sand, granite, and gravel, placed in parallel and capable of removing particulate matter larger than 10 µm prior to treatment by two alternating sodium softeners (III) that decrease water hardness by exchanging calcium and magnesium ions with sodium ions.

Once softened, the water passes through an activated charcoal filter (IV and V) that removes chloride from the water, to prevent damage to the reverse osmosis membranes.The water flows through a 5 µm filter that retains impurities and reaches the reverse osmosis unit, where the water is tangentially forced, by pressure, to cross the membranes (VI), which remove approximately 97% of dissolved

solids, bacteria, endotoxins and organic matter. The following step is the passage of the water through two parallel columns of deionizer (VII), which are mixed anion- and cation-exchange resin beds, to remove the ions that have not been retained by the reverse osmosis membranes. The water is stored in a 1,500 L capacity tank (VIII) provided with a pressure relief that eliminates system overpressure and a spray ball that keeps the inner walls of the tank constantly wet to prevent the bacterial growth. The water is kept in continuous circulation in the looping, flowing close to the 254 nm ultraviolet light source (IX) to prevent microbial proliferation, and through three microbiological 0.05µm filters (X) to retain any microbial cell or debris, and returns to the tank or the purified water is deviated to be distributed to the specific sinks (XI, leakage test place, XII, automatic washer, and XIII, washing components basin) to wash the oxygenator components that are assembled for cardiovascular surgery. At previously established intervals, each sampling point (from I to XIII, Table 1) was sanitized with 70% filtered alcohol. The water flew for one minute and 100 mL water samples were directly packed in sterile polyethylene Whirl-Pak® bags (Nasco, Modesto, CA, USA).

Microbial total counts

Total coliform viable counts were enumerated in minimal R2A agar[1] and m-Endo agar medium, where the coliform colonies exhibit a pink to dark red color, with a green-metallic shine. All media were obtained from Difco (Sparks, MD, USA). A membrane filtration method was used. Each two samples of 100 mL water from each point of the system was filtered through 0.45 µm membrane, which was placed on the surface of R2A agar or on the surface of m-Endo agar. Colony-forming units (CFU/100 mL) were counted after culture at 35.0±0.5ºC for 24 hours. The colony forming units (CFU) present on the membrane surfaces were counted (max. 300 CFU).The Brazilian legislation determines that the counting of heterotrophic bacteria in drinking water doesn't have to exceed the maximum limit of 500 CFU/mL and must have total absence of Escherichia coli or thermo-tolerable coliforms in 100mL of water sample[14]. In the United States Pharmacopoeia, the heterotrophic count bacteria of purified water are required to be less than 100 CFU/mL.

Identification analysis

To identify colonies from the membrane grown on R2A agar, the colonies were streaked onto TSA agar surface and incubated at 35±2°C/24 h. The biochemical identification kits were used after Gram staining and morphological appearance (*coccus* or *bacillus*) of the cells from the isolated grown colonies and subjecting them to oxydase and indol tests. The identification tests for genera and species were performed by using the BBL™ Crystal™ (Becton, Dickinson and Company, Sparks, MD, USA) standardized micro-method systems for identification of Gram-negative (BBL™ Crystal™ Enteric/Non Fermenter panel) and Gram-positive bacteria (BBL™ Crystal™ Gram-positive panel). The interpretation of the reading panel profiles is compared with profiles standards of the data base of software for BBL™ Crystal™ ID system.

pH value and conductivity readings

The pH reading is performed with Metrohm model 744 (Oberdorfstr, Herisau, Switzerland), a potentiometer instrument (pH meter) capable of reproducing pH values to 0.02 pH unit. The conductivity is measured by a conductometer Orion model 5 (Beverly, MA, USA). Due to the temperature have a substantial impact in the conductivity readings, this instrument have an automatic system of compensation of temperature, in order to indicate the real value that theoretically would be observed in the nominal temperature of 25ºC.

Cleaning and sanitization

Cleaning and sanitization of the purification system stages were performed periodically, or when the system was stopped longer than 24 hours for: (i) operational maintenance or (ii) adjustment of the system when unsatisfactory microbial or chemical results were obtained[15]. The cleaning and sanitation procedures are described in Table 2. The reverse osmosis membranes were cleaning initially with a 0.4% sodium hydroxide solution, for 30 minute contact, followed by other 30 minute contact with 2.2% citric acid solution, and finally subjected to the sanitization procedure (Table 2) with 1% Proxitane1512™ (peracetic acid, hydrogen peroxide and acetic acid) solution for 180 minutes.

Table 2: Cleaning and sanitation procedures of each stage of water purification system

Stages	Components	Sampling points	Chemical agents solutions	Contact time
Pretreatment	Potable water	I	-	-
	Multimedium filter	II	Sodium Bissulfite 1%	90 min
	Softener	III	Sodium Bissulfite 1%	90 min
	Charcoal filter	IV and V	Sodium Bissulfite 1%	90 min
	5µm filter	-	-	-
Treatmant	Reverse osmosis	VI	Proxitane® (peracetic acid, hydrogen peroxide and acetic acid) 1%	180 min
	Deionizer	VII	-	-
	Storage tank	VIII	Sodium Hippochlorite 0,4%	240 min
Storage tank	UV lamp	IX	-	-
Distribution	0.05µm filter	X	Sodium Hippochlorite 0,4%	240 min
	Consumption points	XI, XII and XIII	Sodium Hippochlorite 0,4%	240 min

Results and Discussion

This study monitored the water purification system in order to assess the microbiological quality of the water produced, by identifying the critical points of contamination, due to the loss of operating capacity of the system components. The efficacy of the cleaning and sanitation procedures applied to the system was also evaluated. The microbial and chemical analyses by the sampling points were withdrawn from the system for 48 months. In sample 1, after complete sanitization of the purification system, the microbial load brought with the inlet municipal water (I , 1.60 $\log_{10}$) was raised to 2.26 $\log_{10}$ in the charcoal filter (IV and V) and was reduced one decimal log (1.68 $\log_{10}$) by the reverse osmosis membranes. From this step on, the microbial load was reduced to 0.30 $\log_{10}$ in subsequent steps (VII, VIII, IX, X, XI, XII, and XIII), with this value kept to the point of use. The complete sanitization of the system decrease to one decimal log the microbial load in the deionizer (VII) and subsequent steps. However, the sanitization was not efficient between the water inlet and the charcoal filter, as shown in Figure 1 and 2.

In figures 1 and 2 was observed that in samples 1, 2, 4, 5, 6 and 7, after sanitization in the pre-treatment (steps I - V), the spread of disaggregated microorganisms for the next stage happened. However in the samples 3, 8 and 9 verified that in the pre-treatment after sanitization the bioburden stayed equal or was reduced. It was observed that after charcoal filter (V) disinfection, there was a bioburden increment, showing that charcoal filter retained the microorganisms

During the treatment (steps VI and VII), it was verified that the reverse osmosis eliminated the most part of microorganisms, although in the deionizer (VII) occurred a raise of the bioburden. The bacteria, isolated in water samples, from different treatment stages, were submitted for evaluation and identification. A variety of bacteria has been identified in the pre-treatment stage after the inlet water system that spread throughout the system. The species *Flavimonas oryzihabitans* (*Pseudomonas oryzihabitans*) was identified in all stages of the system, even after sanitizing procedures, indicating the possible formation of biofilm, caused by this agent, as shown in Table 3.

The microorganisms that cause infection could be resistant to immune defense mechanisms. The currently used therapeutic agents fail to eradicate systemic infections because have limited spectrum activity. Furthermore, infections caused by biofilms develop resistance to antimicrobials. Therefore, improvement of a better strategy to prevent implant associated infections is an urgent need [16]

Species of *Pseudomonas Chryseobacterium, Acinetobacter, Myroides odoratus, Bergeyella zoohelcum, Lactococcus* have been related to many infections such as bacteremia, urinary tract infection, wound infection, abscess, conjunctivitis, endocarditis, clutter on continuous ambulatory peritoneal dialysis, meningitis and infections associated with invasive devices in debilitated patients [17-21].

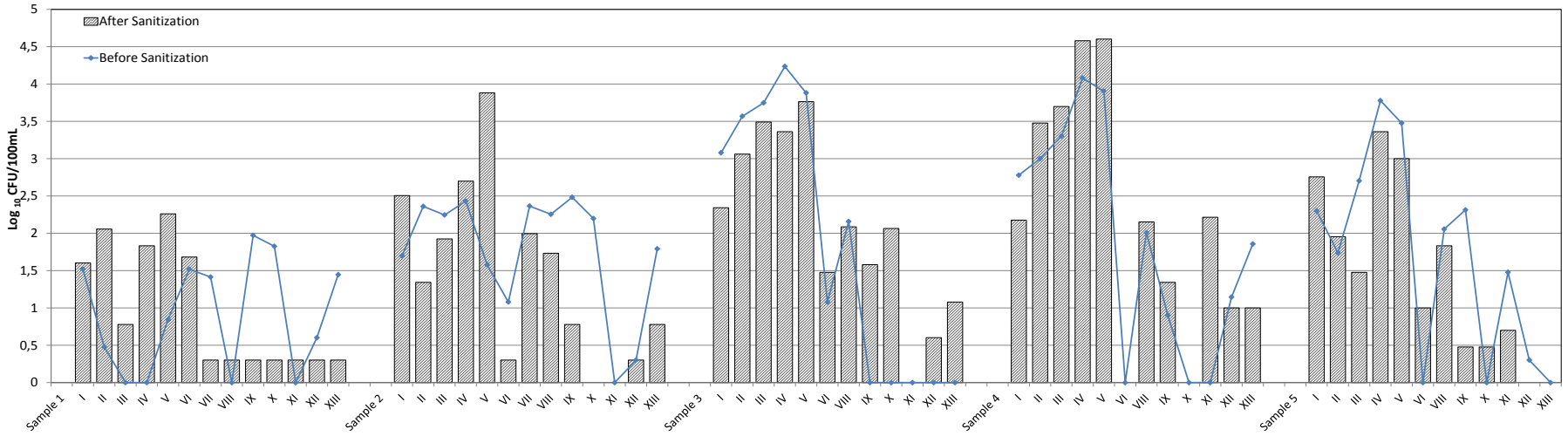

Figure 1: Heterotrophic logarithmic counts related to the purification stages (I – water inlet, II – multimedia filters, III – softeners, IV and V – charcoal filter, VI – reverse osmosis, VII – deionizer, VIII – storage tank, IX – ultraviolet lamp, X – 0.05µm filters, XI – Leakage test, XII - automatic washer, XIII – wash basin) after and before sanitization from samples 1-5

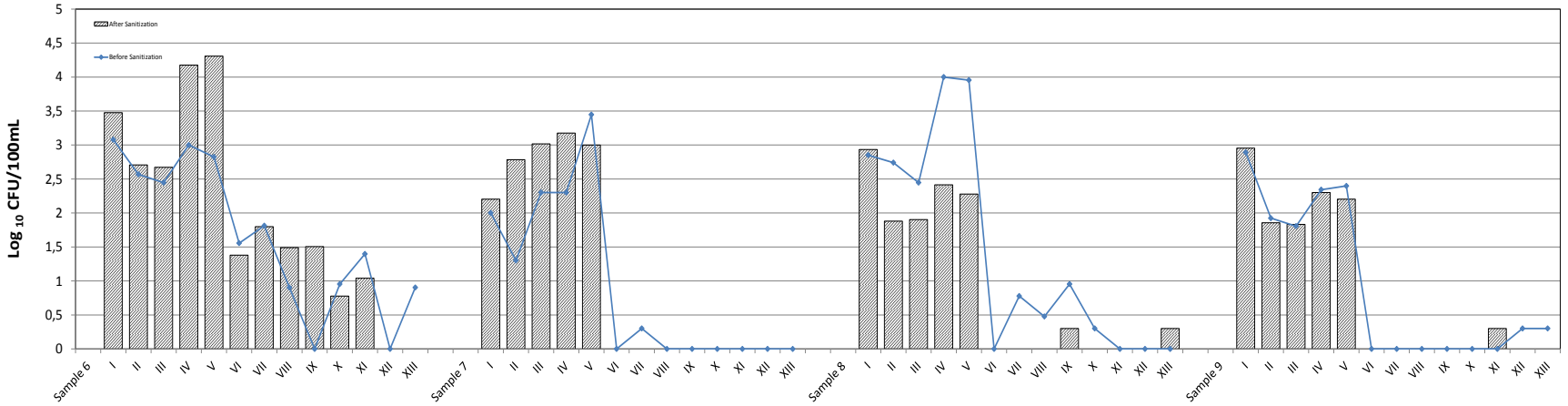

Figure 2: Heterotrophic logarithmic counts related to the purification stages (I – water inlet, II – multimedia filters, III – softeners, IV and V – charcoal filter, VI – reverse osmosis, VII – deionizer, VIII – storage tank, IX – ultraviolet lamp, X – 0.05µm filters, XI – Leakage test, XII - automatic washer, XIII – wash basin) after and before sanitization from sample 6-9

Table 3: Identified bacteria in water in different stages of the system (I, II, III, IV,V,VI)

Stages	Heterotrophic identified bacteria				
I Municipal water	*Flavimonas oryzihabitans*	*Weeksella virosa* *Bergeyella zoohelcum*	*Leifsonia aquaticum*		
II Multimedium Filter	*Flavimonas oryzihabitans*	*Acinetobacter lwoffii*	*Pediococcus species*	*Micrococcus luteus*	*Chryseobacterium indologenes*
III Softner	*Flavimonas oryzihabitans*	*Acinetobacter lwoffii*	*Pediococcus species*	*Gardnerella vaginalis*	*Chryseobacterium indologenes*
IV e V Charcoal Filter	*Flavimonas oryzihabitans*	*Acinetobacter lwoffii*	*Pediococcus species*	*Pseudomonas putida*	*Chryseobacterium indologenes*
VI Reverses Osmosis	*Myroides odoratus*				
VII Deionizer	*Flavimonas oryzihabitans*	*Corynebacterium renale group*			
VIII Storage Tank	*Myroides odoratus*				
IX UV lamp	*Lactococcus raffinolactis*	*Micrococcus luteus*			
X Filter 0,05					
XI Consumption					
XII Consumption	*Flavimonas oryzihabitans*				

The factors that improve bacterial growth in water purification system are "dead spot", areas with poor water circulation and activated charcoal filter, which contribute to the formation of biofilms extremely difficult to be eradicated chemically or physically.Gram-negative bacteria are the most frequently cited contaminants, followed by some Gram-positive bacteria found in water hemodialysis. *Pseudomonas spp* are bacteria most commonly found. *Pseudomonas aeruginosa, Pseudomonas maltophilia* and *Pseudomonas vesicularis* cultures were found in blood of patients with these microorganisms and pyrogenic reactions were also isolated from tap water and dialyzers.

In a study of the microbiological quality of water and dialysate in hemodialysis centers in Greece, Arvanitidou[22]. isolated total coliforms, fecal coliforms, *Pseudomonas* spp., *Streptococcus* and said that these microorganisms should not usually be found in the treated water or dialyses, because it represents a additional risk factor for patients undergoing dialysis [22]. Vorbeck-Meister (1999) [23] examined the water and samples of the row after each step of the water treatment system for dialysis and did not observe any fecal bacteria. Found *P. aeruginosa* at the entrance to a dialysis machine and *P. aeruginosa* and *Enterococcus* output of the dialysis machine.

Corynebacterium was isolated from clinical material and associated with infections such as septicemia, peritonitis, eye infection, wound infection, endocarditis, osteomyelitis, meningitis and abscesses. The leifsonia is also called *Corynebacterium aquaticum*[24] .The species of the genus *Gardnerella* infection is associated with the species *G. vaginalis*, commonly associated with bacterial vaginosis, intrauterine and neonatal sepsis[25]. *Pediococcus* is associated with bacteremia, abscess and infection of pulmonary debilitated patients[26]. *Micrococcus* is a common species in the skin flora. It is associated with bacteremia, endocarditis and septic arthritis [27]. The pH and conductivity readings were taken at the point of consumption of purified water (XI, XII and XIII) and the results of these measurements for 48 months showed average semi conductivity ranging between 0.8 and 1.3 mS/cm^2, values semiannual average pH between 5.6 and 6.1 and results approved verification tests for the presence of oxidizable substances.

Next Steps

Because of clean water disposal during different hemodialysis water treatment (way and sequence). During the osmosis reverse process there is around 30-40% of waste water. The distillatory produces around 80% of wastewater, that is lost in the laboratory. The recycle of these waste water by an integrated pilot unit for water benefited can eliminated the chlorination, the charcoal and reduce the potable water needs for various applications. The recycling clean sources wastewater reduces the demand of potable water provided by Municipal Water Supply (SABESP).The potable water loss is 25% within the campus of USP-Butantã determined by the Program for Rational Use of Water, University of São Paulo (USP-PURA), which monitors the consumption of potable water USP. In 2009 the Basic Sanitation Company of the State of São Paulo created the Corporate Program for Reducing Water Loss that has the goal to reduce the current potable water loss percentage from 25.6% to 15% until 2020.

All water from any source can be treated and processed and be used for various purposes. The water beneficiation is considered the way of purifying water from any impurity degree to a purity degree that is suitable for the intended use, predominantly the importance of selecting and combining many unit processes which are suitable.The Laboratory of Biochemical and Pharmaceutical Technology, College of Pharmaceutical Science, University of São Paulo (FCF-USP) is concerned about the water waste generated by distillers and reverse osmosis, and is looking for solutions for rational use of water, including collecting rainwater. The College of Pharmaceutical Science, University of São Paulo (FCF-USP), consisting of 08 buildings and a portion of Semi-Industrial building is located at the west region of São Paulo, capital of São Paulo state, Brazil. At latitude 23 º 33 '50.63 "S and longitude 46 º 43' 24.34" W, at an altitude of 755m. The rainwater collection in the annual FCF-USP eight buildings, including semi-industrial building (total area of 9.400m$^{2)}$.

The annual average rain was 1,723.3 mm between 2007 to 2012 (data from IAG / USP). Therefore, the annual average rainwater that can be collected in FCF results in the total volume of 16.199.020L/year or 16,199.0m^3/year. Considering only the coverage area of the semi-industrial (3000m^2), annual rainfall average of 1,723.3mm, the total average water uptake corresponds to 5.169.900L/year or 5169.9m^3/year or 430.8m^3/month. The monthly water consumption in this building, corresponds approximately to 400m^3/month. Hence, only the rainwater reuse would be enough to supply the monthly water consumption of the building. The waste water produces by the distiller unit is approximately 150L/h to provide 10L/h distilled water when used for 6 hours/day, that corresponding 900L/day or 18,000L/month or 4.5% water consumed in the building. Therefore the distiller waste water recycling would be enough to supply the water for laboratory needs.The mixture of rain water, waste water from distiller and reverse osmosis will be recycled in an integrated system named Pilot Unit Beneficiation Water (WBPU) (Figure 3).

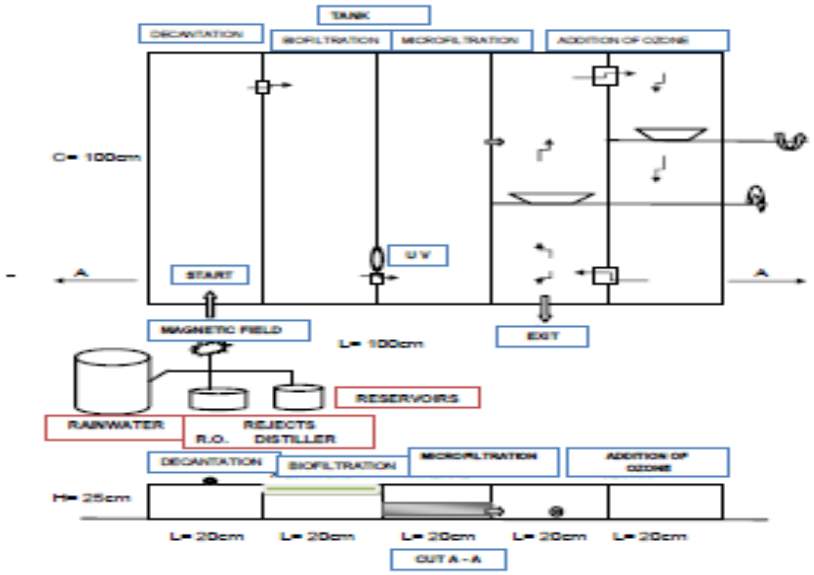

Figure 3: WBPU flowchart

The waste water from different sources will pass through sequential compartments: (a) magnetic yield to salts agglomeration from the water, (b) decantation and (c) biofiltration by aquatic plants to retain: P, F and N (potassium, phosphorus and nitrogen) besides heavy metals, (d) disinfection by ultraviolet radiation, (e) microfiltration with glass microsphere and (f) disinfection process by ozonation (Figure 4).

Figure 4: WBPU picture

Conclusion

Disinfection must be carried out each step of the purification water process, so that there is contamination in the next step. The sanitization of activated charcoal should be made with greater frequency. In Brazil there are still regions where coal is boiled once a week, put in large urban centers coal is sanitized by backwashing with water and 'solution consumption peraceitco 15%. For a more efficient sanitization, the deionizer should be placed before the reverse osmosis and not at the current position and a longer life reverse osmosis.The contamination of water was verified in all the treatment steps for the system studied, especially in the activated carbon (IV and V) one. Then, great contamination was eliminated by R.O. waste water, together with minerals. In order to take the advantage of these waste waters, the WBPU has been proposed. It plans to apply ultraviolet radiation and ozone gas to eliminate contamination from waste waters, rather than the chlorination (R. O. membrane damage). These waste waters can be used to mix with the rain waters and adjust the pH values.

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